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Influence of Valinomycin on Circadian Leaf Movements of *Phaseolus*

(membrane/permeability/circadian rhythms)

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ABSTRACT ***Phaseolus coccineus* was exposed to valinomycin via the transpiration stream for 5-hr periods. The treatment started at various phases of the free-running circadian rhythm in continuous light; it resulted in phase shifts that varied in a manner dependent on the affected phases. The response curves are similar to those for transient withdrawal of water and for light pulses. The results support the hypothesis that membrane processes are important pacemakers in circadian rhythms.**

There is increasing evidence for the role of membranes in circadian rhythms. The influences of heavy water (1), ethyl alcohol (2), and lithium (3) may be mentioned. The hypothesis of the pacemaker function of membrane processes could explain the relative lack of temperature effects on the length of circadian periods.

Circadian changes in permeability are known to occur—for example, water permeability in the case of circadian leaf movements in *Phaseolus* (4), and betacyanin leakage from seedlings of *Chenopodium*—indicating a rhythm in the physical properties of cellular membranes (5). There is also evidence for the role of circadian K^+ fluxes in circadian leaf movements of *Albizza* (6). Since valinomycin causes changes of permeability, especially an increase in the turnover of K^+ we have studied the influence of valinomycin on circadian leaf movements.

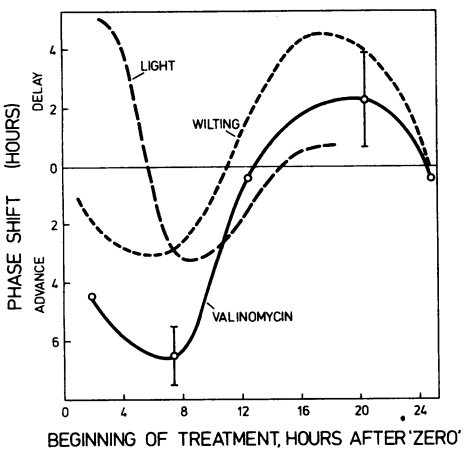

FIG. 1. *Phaseolus coccineus*. Phase shifts of the free-running circadian leaf movements in continuous light due to valinomycin or to other factors. Plants were exposed to valinomycin for one period of 5 hr. The treatment started at various phases of the circadian cycle. "*Zero*" indicates the last subjective night peak of the circadian cycle before treatment. The data refer to delays or advances in the fourth cycle after treatment. Data are compared with earlier published phase-response curves for transient wilting (7), and for pulses of white light (8).

MATERIAL AND METHODS

The plants (*Phaseolus coccineus* = *Ph. multiflorus*) were grown in greenhouses with normal day–night cycles. The experiments were performed with detached plants, which were transferred into growth chambers at constant temperature (21°) and exposed to continuous white light from fluorescent tubes (4,000 lux). Under these conditions, leaf movements reveal a free-running rhythm, with periods of about 28 hr.

Valinomycin (from Calbiochem, San Diego, Calif.) was dissolved in ethyl alcohol, then diluted with distilled water to a final concentration of 10 μg of valinomycin/liter and 10 ml of ethyl alcohol/liter. The plants were exposed to this mixture (valinomycin partially in fine suspension) via the transpiration stream for periods of 5 hr. Treatment was started at various phases of the 28-hr circadian cycle. Control plants were exposed to solutions without valinomycin, but with the same percentage of ethyl alcohol.

RESULTS

Fig. 1 shows the phase-response curve resulting from the application of valinomycin. The data are based on the phase shifts of the fourth cycle after treatment, i.e., the transient cycles were excluded. The figure gives median values from 4–9 single plants. The ranges of standard deviation given at the maximum and minimum of the response curve indicate rather high individual differences. These are probably due to the well-known individual differences in strength of the transpiration stream. Nonetheless, each of the nine single values from which the median value for maximum advance was calculated, was an increase as compared with the controls.

The response curve resembles those for the influence of transient wilting due to reduced water supply (Fig. 1). Furthermore, a transient withdrawal of water resulting from exposure to solutions of sucrose instead of water yielded similar results (unpublished), especially with respect to the phase that reacts with maximum advance.

Fig. 1 also shows some similarities between the response curves for valinomycin effects and for pulses of white light. The response curve for pulses with far-red light (8) is even more similar to the curve for valininomycin effects.

DISCUSSION

The results provide further evidence for the hypothesis that membrane processes are important pacemakers in circadian

rhythms. It is not possible to explain the similarities between the responses caused by transient treatment with valinomycin, by transient withdrawal of water, and by light pulses. However, two analogous similarities can be mentioned. (*i*) Both the light-controlled and the endogenous circadian leaflet movements of *Albizzia* are based on K^+ fluxes (9). (*ii*) The flowering of *Lemma perpusilla* is inhibited in a periodic manner by daily transfer from the nutrient solution to distilled water for short periods of time. The phases of maximal inhibition of flowering coincide partially with the phases of maximal sensitivity to light pulses (10).

1. Enright, J. T. (1971) "Heavy water slows biological timing processes," *Z. Vergl. Physiol.* **72**, 1–16.
2. Enright, J. T. (1971) "The internal clock of drunken isopods," *Z. Vergl. Physiol.* **75**, 332–346.
3. Engelmann, W. (1972) "Lithium slows down the *Kalanchoe* clock," *Z. Naturforsch. B*, **27**, 477.
4. Bünning, E. (1934) "Die Mechanik der tagesperiodischen Variationsbewegungen von *Phaseolus multiflorus*," *Jahrb. Wiss. Bot.* **79**, 191–230.
5. Wagner, E. & Cumming, B. G. (1970) "Betacyanin accumulation, chlorophyll content, and flower initiation in *Chenopodium rubrum* as related to endogenous rhythmicity and phytochrome action," *Can. J. Bot.* **48**, 1–18.
6. Satter, Rl. & Galston, A. W. (1971) "Phytochrome-controlled nyctinasty in *Albizzia julibrissin* III. Interactions between an endogenous rhythm and phytochrome in control of potassium flux and leaflet movement," *Plant Physiol.* **48**, 740–746.
7. Bünning, E. & Moser, I. (1968) "Einfluss des Wassers auf die circadiane Rhythmik von *Phaseolus*," *Naturwissenschaften* **55**, 450–451.
8. Bünning, E. & Moser, I. (1966) "Response Kurven bei der circadianen Rhythmik von *Phaseolus*," *Planta* **69**, 101–110.
9. Satter, R. L. & Galston, A. W. (1971) "Potassium flux: a common feature of *Albizzia* leaflet movement controlled by phytochrome or endogenous rhythms," *Science* **174**, 518–519.
10. Halaban, R. & Hillman, W. S. (1970) "Response of *Lemna perpusilla* to periodic transfer to distilled water," *Plant Physiol.* **46**, 641–644.